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Serial Number: 09/903,410

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Mary Jane Ruhl Tech. Info. Specialist, STIC TC-1600 CM-1, Room 6A-06 Phone: 605-1155

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2	L2	116	aquifex or pyrophilus	USPAT; US-PGPUB	2003/06/06 15:08
3	L3	22	1 and 2	USPAT; US-PGPUB	2003/06/06 15:29
4	L4	5	1 same 2	USPAT; US-PGPUB	2003/06/06 15:29

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PGPUB-DOCUMENT-NUMBER: 20030064491

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030064491 A1

TITLE:

Genes and proteins involved in the biosynthesis of

enediyne ring structures

PUBLICATION-DATE: April 3, 2003

INVENTOR-INFORMATION:

NAME CITY STATE COUNTRY RULE-47

Farnet, Chris M. Outremont CA
Staffa, Alfredo Saint-Laurent CA

Zazopoulos, Emmanuel Montreal CA

APPL-NO: 10/ 152886

DATE FILED: May 21, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60291959 20010521 US

non-provisional-of-provisional 60334604 20011203 US

US-CL-CURRENT: 435/183, 435/320.1, 435/325, 435/69.1, 435/76, 536/23.2

ABSTRACT:

Five protein families cooperate to form the warhead structure that characterizes enediyne compounds, both chromoprotein enediynes and non-chromoprotein enediynes. The protein families include a polyketide synthase and thioesterase protein which form a polyketide synthase catalytic complex involved in warhead formation in enediynes. Genes encoding a member of each of the five protein families are found in all enediyne biosynthetic loci. The genes and proteins may be used in genetic engineering applications to design new enediyne compounds and in methods to identify new enediyne biosynthetic loci.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 USC .sctn. 119 of provisional applications U.S. Ser. No. 60/291,959 filed on May 21, 2001 and U.S. Ser. No. 60/334,604 filed on Dec. 3, 2001 which are hereby incorporated by reference in their entirety for all purposes.

----- KWIC -----

Detail Description Table CWU - DETL (11):

11TABLE 10 135E locus GenBank homology proposed function of Family #aa Accession, #aa probability identity similarity GenBank match PKSE 1933 T37056, 2082aa 1e-65 282/909 (31.02%) 365/909 (40.15%) multi-domain beta keto-acyl synthase, Streptomyces coelicolor BAB69208 1, 2365aa 3e-84 285/925 (30.81%) 366/925 (39.57%) polyketide synthase, Streptomyces avermitilis T30937, 1053aa 2e-69 246/907 (27.12%) 356/907 (39.25%) glycolipid synthase, Nostoc punctiforme TEBC 154 NP_249659 1, 146aa 2e-07 41/132 (31.06%) 63/132 (47.73%) hypothetical protein, Pseudomonas aeruginosa AAD49752 1, 148aa 2e-06 40/132 (30.3%) 62/132 (46.97%) orf1, Pseudomonas aeruginosa NP_214031 1, 128aa 5e-04 35/127 (27.56%) 60/127 (47.24%) hypothetical protein, Aquifex aeolicus UNBL 323 NO HOMOLOG UNBV 655 CAC44518 1, 706aa 9e-04 41/135 (30.37%) 59/135 (43.7%) putative secreted esterase, Streptomyces coelicolor UNBU 346 NP_486037 1, 300aa 4e-09 52/191 (27.23%) 87/191 (45.55%) hypothetical protein, Nostoc sp NP_440874 1, 285aa 9e-06 47/197 (23.86%) 89/197 (45.18%) hypothetical protein, Synechocystis sp

PGPUB-DOCUMENT-NUMBER: 20030054530

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030054530 A1

TITLE:

Esterases

PUBLICATION-DATE:

March 20, 2003

INVENTOR-INFORMATION:

NAME CITY STATE COUNTRY RULE-47

Robertson, Dan E. Solana Beach CA US US Murphy, Dennis Malvern PA Reid, John Ardmore PA US Maffia, Anthony M. Old Bridge US NJ Link, Steven Wilmington DE US Swanson, Ronald V. Del Mar CA US Warren, Patrick V. Coatesville PA US Lenox, Anna Perkiomenville PA US Short, Jav M. Rancho Santa Fe CA US

Mathur, Eric J.

Carlsbad CA US

APPL-NO: 10/027804

DATE FILED: December 21, 2001

RELATED-US-APPL-DATA:

child 10027804 A1 20011221

parent division-of 09903410 20010710 US PENDING

child 09903410 20010710 US

parent continuation-in-part-of 09382242 19990824 US PENDING

child 09382242 19990824 US

parent continuation-of 08602359 19960216 US GRANTED

parent-patent 5942430 US

US-CL-CURRENT: 435/196

ABSTRACT:

Esterase enzymes derived from various Staphylothermus, Pyrodictium, Archaeoglobus, Aquifex, M11TL, Thermococcus, Teredinibacter and Sulfolobus organisms are disclosed. The enzymes are produced from native or recombinant host cells and can be utilized in pharmaceutical, agricultural and other

industries.
RELATED APPLICATIONS
[0001] This application is a divisional of co-pending U. S. patent application Ser. No. 08/602,359, filed Feb. 17, 1996.
KWIC

Abstract Paragraph - ABTX (1):

Esterase enzymes derived from various Staphylothermus, Pyrodictium, Archaeoglobus, **Aguifex**, M11TL, Thermococcus, Teredinibacter and Sulfolobus organisms are disclosed. The enzymes are produced from native or recombinant host cells and can be utilized in pharmaceutical, agricultural and other industries.

PGPUB-DOCUMENT-NUMBER: 20020164725

PGPUB-FILING-TYPE:

new

DOCUMENT-IDENTIFIER: US 20020164725 A1

TITLE:

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Esterases

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

NAME CITY STATE COUNTRY RULE-47

Robertson, Dan E. Solana Beach NJ US Murphy, Dennis Malvern PA US Reid, John Armore US PA Maffia, Anthony Wilmington DE US Link, Steven Wilmington DE US Swanson, Ronald V. Del Mar CA US Warren, Patrick V. Coatesville PA US Kosmatka, Anna US Doylestown PΑ

APPL-NO:

10/027805

DATE FILED: December 21, 2001

RELATED-US-APPL-DATA:

child 10027805 A1 20011221

parent division-of 09903410 20010710 US PENDING

child 09903410 20010710 US

parent continuation-in-part-of 09382242 19990824 US PENDING

child 09382242 19990824 US

parent continuation-of 08602359 19960216 US GRANTED

parent-patent 5942430 US

US-CL-CURRENT: 435/106

ABSTRACT:

Esterase enzymes derived from various Staphylothermus, Pyrodictium, Archaeoglobus, Aquifex, M11TL, Thermococcus, Teredinibacter and Sulfolobus organisms are disclosed. The enzymes are produced from native or recombinant host cells and can be utilized in pharmaceutical, agricultural and other industries.

RELATED APPLICATIONS

[0001] This application is a divisional of co-pending U.S. patent application Ser. No. 08/602,359, filed Feb. 17, 1996.	
KWIC	

Abstract Paragraph - ABTX (1):

<u>Esterase</u> enzymes derived from various Staphylothermus, Pyrodictium, Archaeoglobus, <u>Aquifex</u>, M11TL, Thermococcus, Teredinibacter and Sulfolobus organisms are disclosed. The enzymes are produced from native or recombinant host cells and can be utilized in pharmaceutical, agricultural and other industries.

US-PAT-NO:

6562958

DOCUMENT-IDENTIFIER: US 6562958 B1

TITLE:

٠.

Nucleic acid and amino acid sequences relating to Acinetobacter baumannii for diagnostics and therapeutics

DATE-ISSUED:

May 13, 2003

INVENTOR-INFORMATION:

NAME

CITY

STATE ZIP CODE COUNTRY

Breton; Gary

Marlborough

MA

N/A

Bush; David

Somerville

MA

N/A N/A

N/A

APPL-NO:

09/328352

DATE FILED: June 4, 1999

PARENT-CASE:

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/088,701, filed Jun. 9, 1998, the entire teachings of which are incorporated herein by reference.

US-CL-CURRENT: 536/23.7, 536/23.1

ABSTRACT:

The invention provides isolated polypeptide and nucleic acid sequences derived from Acinetobacter mirabilis that are useful in diagnosis and therapy of pathological conditions; antibodies against the polypeptides; and methods for the production of the polypeptides. The invention also provides methods for the detection, prevention and treatment of pathological conditions resulting from bacterial infection.

15 Claims, 0 Drawing figures

Exemplary Claim Number:

----- KWIC -----

Detailed Description Paragraph Table - DETL (77):

AMINOBENZOATE SYNTHASE COMPONENT I, (ADC SYNTHASE)] [SP:P05041] Contig140G 1361583 c1 62 1857 5983 696 231 468 1.90E-44 sp:[LN:HISI BACSU] [AC:034520] [GN:HISG] [OR:BACILLUS SUBTILIS] [EC:2.4.2.17] [DE:ATP PHOSPHORIBOSYLTRANSFER ASE,] [SP:034520] Contig140G 1443927 c1 72 1858 5984

```
681 226 343 3.30E-31 sp:[LN:YEAZ ECOLI] [AC:P76256:O08476:O08477] [GN:YEAZ]
[OR:ESCHERICHIA COLI] [DE:HYPOTHETICAL 25.2 KD PROTEIN IN FADD- PABB
INTERGENIC REGION] [SP:P76256:O08476:O08477] Contig140G 19800062_f1_9 1859
5985 591 196 425 6.70E-40 gp:[GI:g1871177] [LN:ATU90439] [AC:U90439]
[GN:T06D20.4] [OR:Arabidopsis thaliana] [SR:thale cress] [DE:Arabidopsis
thaliana chromosome II BAC T06D20 genomic sequence, complete sequence.]
[NT:unknown protein] Contig140G 20744002 c2 85 1860 5986 534 177 481 7.80E-46
sp:[LN:FBP_PSEAE] [AC:P40882] [GN:FBP] [OR:PSEUDOMONAS AERUGINOSA]
[DE:FERRIPYOCHELIN BINDING PROTEIN] [SP:P40882] Contig140G 22689193_c3_97
1861 5987 1677 558 80 9.30E-05 pir:[LN:H70355] [AC:H70355] [PN:hypothetical
protein aq_627] [GN:aq_627] [OR:Aquifex acolicus] Contig140G 23703138 c2_88
1862 5988 849 282 581 2.00E-56 pir:[LN:F64819] [AC:F64819] [PN:hypothetical
protein b0822] [OR:Escherichia coli] Contig140G 23853200_c2_90 1863 5989 837
278 223 1.80E-18 pir:[LN:B36868] [AC:B36868] [PN:copB
homolog:hypotheticalprotein 2] [OR:Xanthomonas campestris] Contig140G
24407827 c3 99 1864 5990 1926 641 NO-HIT Contig140G 24431540 c3 102 1865 5991
843 280 294 5.10E-26 sp:[LN:YHIQ NEIGO] [AC:P72077] [OR:NEISSERIA
GONORRHOEAE] [DE:HYPOTHETICAL 27.3 KD PROTEIN] [SP:P72077] Contig140G
24662562_c2_91 1866 5992 336 111 301 9.30E-27 sp:[LN:SUGE_CITFR] [AC:O69279]
[GN:SUGE] [OR:CITROBACTER FREUNDII] [DE:SUGE PROTEIN HOMOLOG] [SP:O69279]
Contig140G 258442 c1 76 1867 5993 1056 352 1240 2.90E-126 sp:[LN:NADA ECOLI]
[AC:P11458:P77373] [GN:NADA:NICA] [OR:ESCHERICHIA COLI] [DE:QUINOLINATE
SYNTHETASE A] [SP:P11458:P77373] Contig140G 26259753_c2_79 1868 5994 273 90
373 2.20E-34 sp:[LN:YRPM_ACICA] [AC:P33989] [OR:ACINETOBACTER
CALCOACETICUS] [DE:HYPOTHETICAL 9.2 KD PROTEIN IN RPON-MURA INTERGENIC
REGION (ORF3)] [SP:P33989] Contig140G 26350790_c1_63 1869 5995 1353 450 1024
2.20E-103 pir:[LN:E70368] [AC:E70368] [PN:histidinol dehydrogenase]
[GN:hisD] [CL:histidinol dehydrogenase:histidinol dehydrogenase homology]
[OR: Aquifex aeolicus] Contig140G 26579061 c2 93 1870 5996 1242 413 1013
3.30E-102 sp:[LN:ARGJ NEIGO] [AC:P38434] [GN:ARGJ] [OR:NEISSERIA
GONORRHOEAE] [EC:2.3.1.35:2.3.1.1] [DE:ACETYLTRANSFERASE,
(N-ACETYLGLUTAMATE
SYNTHASE)(AGS)] [SP:P38434] Contig140G 26600052_c2 80 1871 5997 1263 420 1928
3.60E-199 sp:[LN:MURA_ACICA] [AC:P33986] [GN:MURA:MURZ] [OR:ACINETOBACTER
CALCOACETICUS] [EC:2.5.1.7] [DE:TRANSFERASE)(EPT)] [SP:P33986] Contig140G
2853452_c3_104 1872 5998 399 132 NO-HIT Contig140G 29485012_c3_106 1873 5999
1032 343 174 8.50E-13 gp:[GI:e258655:g1628369] [LN:DNINTREG] [AC:X98546]
[GN:gepB] [OR:Dichelobacter_nodosus] [DE:D.nodosus intB, regA, gepA, gepB,
and gepC genes.] Contig140G 30704408_c1_75 1874 6000 573 190 356 1.40E-32
gp:[GI:e1370607:g4158208] [LN:SC9B5] [AC:AL035206] [PN:putative
methylated-DNA- protein-cysteine] [GN:SC9B5.29] [OR:Streptomyces coelicolor]
[DE:Streptomyces coelicolor] cosmid 9B5.] [NT:SC9B5.29, ogt2,
methylated-DNA-protein- cysteine] Contig140G 31822052_c3_98 1875 6001 192 63
112 9.90E-07 pir:[LN:S66936] [AC:S66936:S662927] [PN:probable membrane
protein YOR053w:hypothetical protein O2799] [OR:Saccharomyces cerevisiae]
[MP:15R] Contig140G 33618802 c2 87 1876 6002 882 293 725 1.10E-71
sp:[LN:ESTD_HUMAN] [AC:P10768] [GN:ESD] [OR:HOMO SAPIENS] [EC:3.1.1.1]
[DE:ESTERASE D,] [SP:P 0768] Contig140G 35267331 c1 74 1877 6003 2016 671
1238 4.70E-126 pir:[LN:A36868] [AC:A36868] [PN:copA homolog:hypothetical
protein 1] [CL:laccase] [OR:Xanthomonas campestris] Contig140G 35781253 f1 10
1878 6004 261 86 119 1.80E-07 pir:[LN:S56703] [AC:S56703] [PN:glycine-rich
cell wall protein precursor:CEM6 protein] [OR:Daucus carota] [SR:, carrot]
Contig140G 35948293_c3_95 1879 6005 867 288 128 6.80E-08 pir:[LN:H69061]
```

Detailed Description Paragraph Table - DETL (141):

[AC:P37764] [GN:YAEL] [OR:ESCHERICHIA COLI] [DE:HYPOTHETICAL 49.1 KD PROTEIN IN CDSA-HLPA INTERGENIC REGION] [SP:P37764] Contig151G 4101588_f3_442 3347 7473 1062 353 941 1.40E-94 gp:[GI:d1033097:g3401952) [LN:AB011413] [AC:AB011413] [PN:Orf8] [FN:alcohol dehydrogenase] [OR:Streptomyces griseus] [SR:Streptomyces griseus DNA] [DE:Streptomyces griseus genes for Orf2, Orf3, Orf4, Orf5, AfsA, Orf8, partial and complete cds.] Contig151G 4103461 c3_1036 3348 7474 897 298 318 1.50E-28 gp:[GI:g3660461] [LN:PSAF001355] [AC:AF001355:U16026:U03465: U87170] [PN:DNA binding protein HpkR] [GN:hpkR] [OR:Pseudomonas syringae pv. syringae] [DE:Pseudomonas syringae pv. syringae DNA binding protein HpkR (hpkR),hybrid histidine protein kinase-phosphate acceptor regulatoryprotein CvgSY (cvgSY), ankyrin AnkF (ankF), and catalase isozymecatalytic subunit CatF (catF) genes, complete cds.] [NT:similarity suggests this is a member of the HTH] Contig151G 4103462 c2 724 3349 7475 1581 526 351 2.30E-30 gp:|Gl:g3414726| [LN:AF047693] [AC:AF047693] [PN:multidrug resistance efflux pump homolog PmrB] [GN:pmrB] [OR:Pseudomonas aeruginosa] [DE:Pseudomonas aeruginosa multidrug resistance efflux pump homologPmrA (pmrA) and multidrug resistance efflux pump homolog PmrB(mmrB) genes, complete cds.] [NT:14 TMS efflux pump; similar to EmrB of Escherichia] Contig151G 4105312_f3_402 3350 7476 375 124 NO-HIT Contig151G 4110142_c2_814 3351 7477 558 185 120 1.40E-07 gp:[Gl:g4139249] [LN:AF110185] [AC:AF110185] [PN:unknown] [OR:Burkholderia pseudomallei] [DE:Burkholderia pseudomallei strain 1026b DbhB (dbhB). general secretory pathway protein D (gspD), general secretory pathway protein E (gspE), general secretory pathway protein F (gspF), GspC(gspC), general secretory pathway protein G (gspG), generalsecretory pathway protein H (gspH), general secretory pathwayprotein I (gsp1), general secretory pathway protein J (gspJ),general secretory pathway protein K (gspK), general secretorypathway protein L (gspL), general secretory pathway protein M(gspM), and general secretory pathway protein N (gspN) genes, complete cds; and unknown genes.] [NT:similar to Escherichia coli MarR protein; orfE] Contig151G 4118800_f1_63 3352 7478 1104 367 304 4.50E-27 gp:[GI:g2852632] [LN:AF007152] [AC:AF007152] [PN:unknown] [OR:Homo sapiens] [DE:Homo sapiens clone 23649 and 23755 unknown mRNA, partial cds.] Contig151G 4140712_c2_739 3353 7479 336 111 114 6.10E-07 sp:[LN:YH83_SYNY3] [AC:P73602] [GN:SLL1783] [OR:SYNECHOCYSTIS SP] [SR:PCC 6803,] [DE:HYPOTHETICAL 16.8 KD PROTEIN SLL1783] [SP:P73602] Contig151G 4142840 f2 257 3354 7480 528 175 NO-HIT Contig151G 4147318_c2_733 3355 7481 1350 449 923 1.10E-92 sp:[LN:TUB3 AGRVI] [AC:P70786] [GN:TTUB] [OR:AGROBACTERIUM VITIS] [DE:PUTATIVE TARTRATE TRANSPORTER] [SP:P70786] Contig151G 4156377_c2_174 3356 7482 891 296 877 8.50E-88 pir:[LN:E69778] [AC:E69778] [PN:conserved hypothetical protein ydeK] [GN:ydeK] [OR:Bacillus subtilis] Contig151G 42087_f1_88 3357 7483 858 285 1169 970E-119 pir:[LN:JC4161] [AC:JC4161:PC4038] [PN:probable chloride peroxidase,:esterase (misidentification)] [CL:peroxidase] [OR:Pseudomonas putida] [EC:1.11.1.10] Contig151G 4335950 f2 156 3358 7484 1155 384 138 5.40E-07 pir:[LN:E70470] [AC:E70470] [PN:conserved hypothetical protein aq_1986] [GN:aq_1986] [OR:Aquifex aeolicus] Contig151G 4339132 c1 621 3359 7485 639 212 133 1.50E-07 pir:[LN:H65092] [AC:H65092] [PN:hypothetical protein b3050] [OR:Escherichia coli] Contig151G 4344568 c1 568 3360 7486 816 271 337 1.40E-30 pir:[LN:S69588] [AC:S69588] [PN:hypothetical protein

YDR533c] [CL:conserved hypothetical protein YMR322c] [OR:Saccharomyces cerevisiae] [MP:4R] Contig151G 4375258_c3_994 3361 7487 1464 487 1670 7.90E-172 gp:[GI:e321556:g2208982] [LN:YEY13308] [AC:Y13308] [PN:sulfate permease] [OR:Yersinia enterocolitica] [DE:Yersinia enterocolitica plasmid DNA fragment, strain 15673.] [NT:ORF3] Contig151G 439452_f1_57 3362 7488 792 263 NO-HIT Contig151G 4398376_f1_335 3363 7489 513 170 NO-HIT Contig151G 4429088_f2_268 3364 7490 468 155 NO-HIT Contig151G 4429642_f3_419 3365 7491 1269 422 601 1.50E-58 gp:[GI:g2291144] [LN:CELF17A9] [AC:AF016417] [GN:F17A9.4]

US-PAT-NO:

5942430

DOCUMENT-IDENTIFIER: US 5942430 A

TITLE:

Esterases

DATE-ISSUED:

August 24, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CO	DE COUN	ITRI
Robertson; Dan E.	Haddonfield	NJ	N/A	N/A	
Murphy; Dennis	Paoli	PA	N/A	N/A	
Reid; John	Bryn Mawr	PA	N/A	N/A	
Maffia; Anthony M.	Wilmington	DE	N/A	N/A	
Link; Steven	Wilmington	DE	N/A	N/A	
Swanson; Ronald V.	Media	PA	N/A	N/A	
Warren; Patrick V.	Philadelphia	PA	N/A	N/A	
Kosmotka; Anna	Brookhaven	PA	N/A	N/A	

APPL-NO:

08/602359

DATE FILED: February 16, 1996

US-CL-CURRENT: 435/197, 435/196, 435/252.3, 435/320.1, 435/325, 536/23.2

ABSTRACT:

Esterase enzymes derived from various Staphylothermus, Pyrodictium, Archaeoglobus, Aquifex, M11TL, Thermococcus, Teredinibacter and Sulfolobus organisms are disclosed. The enzymes are produced from native or recombinant host cells and can be utilized in the pharmaceutical, agricultural and other industries.

9 Claims, 17 Drawing figures

Exemplary Claim Number:

Number of Drawing Sheets: 17

----- KWIC -----

Abstract Text - ABTX (1):

Esterase enzymes derived from various Staphylothermus, Pyrodictium, Archaeoglobus, Aquifex, M11TL, Thermococcus, Teredinibacter and Sulfolobus organisms are disclosed. The enzymes are produced from native or recombinant host cells and can be utilized in the pharmaceutical, agricultural and other industries.

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L5 43911 ESTERASE#

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L6 14071 ESTERASE#

FILE 'HCAPLUS'

L7 33241 ESTERASE#

FILE 'NTIS'

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FILE 'LIFESCI'

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L16 23 AQUIFEX OR PYROPHILUS

FILE 'BIOSIS'

206 AQUIFEX

50 PYROPHILUS

L17 210 AQUIFEX OR PYROPHILUS

FILE 'EMBASE'

128 AQUIFEX

29 PYROPHILUS

L18 128 AQUIFEX OR PYROPHILUS

FILE 'HCAPLUS'

255 AQUIFEX

53 PYROPHILUS

L19 255 AQUIFEX OR PYROPHILUS

FILE 'NTIS'

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1 PYROPHILUS

L20 1 AQUIFEX OR PYROPHILUS

FILE 'ESBIOBASE'

125 AQUIFEX

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FILE 'SCISEARCH'

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FILE 'LIFESCI'

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FILE 'BIOTECHDS'

L28 2 L4 AND L16

FILE 'BIOSIS'

L29 0 L5 AND L17

FILE 'EMBASE'

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L30
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 FILE 'HCAPLUS'
              3 L7 AND L19
 FILE 'NTIS'
 L32
              0 L8 AND L20
 FILE 'ESBIOBASE'
 L33
              0 L9 AND L21
 FILE 'BIOTECHNO'
T.34
              0 L10 AND L22
 FILE 'WPIDS'
L35
              1 L11 AND L23
 TOTAL FOR ALL FILES
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PROCESSING COMPLETED FOR L36
               4 DUP REM L36 (3 DUPLICATES REMOVED)
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L37
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      New isolated nucleic acid and its variants comprising specified
TT
      consecutive amino acid sequences and corresponding cDNA sequences, encode
      polypeptides having esterase activity;
          recombinant enzyme production via plasmid expression in host cell, for
          ester hydrolysis
      ROBERTSON D E; MURPHY D; REID J; MAFFIA A M; LINK S; SWANSON R; WARREN P
ΑU
      V; KOSMATKA A
AN
      2003-07813 BIOTECHDS
      US 2002146799 10 Oct 2002
PΙ
     ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2003 ACS
L37
     Nucleic acids encoding human cyclic nucleotide-associated proteins
TI
     PCT Int. Appl., 78 pp.
SO
     CODEN: PIXXD2
IN
     Hillman, Jennifer L.; Yue, Henry; Guegler, Karl J.; Corley, Neil C.;
     Patterson, Chandra; Tang, Y. Tom
ΆN
     2000:175945 HCAPLUS
DN
     132:218013
     PATENT NO.
                      KIND DATE
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PΙ
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     WO 2000014248
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                                           WO 1999-US20287 19990903
         W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
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             MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
             TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU,
             TJ, TM
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             ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG,
             CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
     AU 9960263
                       A1
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                                           AU 1999-60263
                                                              19990903
     EP 1144648
                       Α1
                             20011017
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            AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, SI, LT, LV, FI, RO
L37
    ANSWER 3 OF 4
                       MEDLINE
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Mutational analysis of the RecJ exonuclease of Escherichia coli:

TI

identification of phosphoesterase motifs.

SO JOURNAL OF BACTERIOLOGY, (1999 Oct) 181 (19) 6098-102.

Journal code: 2985120R. ISSN: 0021-9193.

AU Sutera V A Jr; Han E S; Rajman L A; Lovett S T

AN 1999429858 MEDLINE

L37 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

TI New nucleic acid encoding heat stable esterases from

thermophilic bacteria;

recombinant thermostable esterases for use in

pharmaceutical, agricultural or food industries, etc.

AU Robertson D E; Murphy D; Reid J; Maffia A M; Link S; Swanson R V; Warren

P V; Kosmotka A; Callen W

AN 1997-11973 BIOTECHDS

PI WO 9730160 21 Aug 1997

=> d ab tot

L37 ANSWER 1 OF 4 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI DERWENT ABSTRACT:

NOVELTY - Isolated nucleic acid (I) and its variants comprising consecutive amino acid sequences and corresponding cDNA sequences given in the specification, encoding a polypeptide with **esterase** activity, are new.

DETAILED DESCRIPTION - An isolated nucleic acid and its variants encoding a polypeptide with esterase activity comprising sequence of 185 amino acids and corresponding cDNA sequence of 555 bp, 347 amino acids and corresponding cDNA sequence of 1041 bp, 263 amino acids and corresponding cDNA sequence of 789 bp, 252 amino acids and corresponding cDNA sequence of 756 bp, 298 amino acids and corresponding cDNA sequence of 894 bp, 263 amino acids and corresponding cDNA sequence of 789 bp, 250 amino acids and corresponding cDNA sequence of 750 bp, 339 amino acids and corresponding cDNA sequence of 1017 bp, 311 amino acids and corresponding cDNA sequence of 936 bp, or 305 amino acids and corresponding cDNA sequence of 918 bp given in the specification, are new. The variants of the nucleic acid have at least50% identity to the same sequences above. INDEPENDENT CLAIMS are also included for: (1) a computer readable medium storing the specified sequences above as well as specified deduced amino acid sequences; (2) preparing a first sequence to a reference sequence comprising reading the first sequence and reference sequence through the use of computer program which compares the sequences, and determining the differences between the first sequence and reference sequence with the computer program; (3) an assay for identifying functional polypeptide fragments or variants encoded by the fragments of specified sequences above as well as specified deduced amino acid sequences, comprising contacting the polypeptide with a substrate molecule under conditions allowing the polypeptide or fragment or variant to function, and detecting if there is either decrease in the level of substrate or increase in the level of substrate reaction product of the reaction between the polypeptide and substrate; (4) a nucleic acid probe comprising oligonucleotide of 10-50 nucleotides in length and having an area of at least10 contiguous nucleotides that is at least50% complementary to the nucleic acid target region of the nucleic acid with specified sequences; (5) an enzyme preparation comprising the liquid or dry polypeptide; and (6) an antibody that specifically binds to the polypeptide.

BIOTECHNOLOGY - Preparation: The polypeptide is prepared by introducing a nucleic acid encoding the polypeptide into a host cell under conditions that allow expression of the polypeptide and recovering the polypeptide. Its variants are generated by obtaining a nucleic acid comprising the specified sequences of fragments comprising at least30 consecutive nucleotides and complementary to the above sequence, modifying nucleotides in the sequence to another nucleotide, and deleting

or adding one or more sequences in the sequence. Preferred Properties: The isolated nucleic acid hybridizes to nucleic acid under high, moderate or low stringency conditions. The nucleic acid also encodes polypeptide having deduced amino acid sequence of Staphylothermus marinus F1-12LC, deduced amino acid sequence of Pyrodictium TAGI 1-17LC, deduced amino acid sequence of Archaeoglobus venificus SNP6-24LC, deduced amino acid sequence of Aquifex pyrophilus-28LC, deduced amino acid sequence of M11TL-29L, deduced amino acid sequence of Thermococcus CL-2-30LC, deduced amino acid sequence of Aquifex VF5-34LC, deduced amino acid sequence of Teredinibacter-42L, deduced amino acid sequence of Archaeoglobus fulgidus VC16-16MC and deduced amino acid sequence of Sulfolobus solfataricus P1-8LC. The purified polypeptide has at least50 (preferably at least95)% homology to the nucleic acid as determined by analysis with a sequence comparison algorithm. The sequence comparison algorithm is FASTA version 3.0t78 with the default parameters. The antibodies may be polyclonal or monoclonal. The oligonucleotide is DNA. It has 15-50 bases in length. Preferred Components: The probe comprises a detectable isotropic label, detectable non-isotropic label, which is fluorescent molecule, chemiluminescent molecule, enzyme, cofactor, enzyme substrate or hapten. It hybridizes to the nucleic acid target region under moderate to highly stringent conditions to form a detectable target probe duplex. Preferred Method: The modifications are introduced by error-prone polymerase chain reaction (PCR), shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive, site-specific mutagenesis, gene reassembly and/or gene site saturated mutagenesis. The differences between the sequences are determined by identifying the polymorphisms.

USE - (I) is useful as encoding polypeptides having **esterase** activity. The polypeptide is an enzyme that catalyzes the hydrolysis of esters.

ADVANTAGE - The polypeptide has increased **esterase** activity and stability at increased pH temperature. It is stable to heat and is able to renature and regain activity after exposure to 60-105degreesC.

EXAMPLE - DNA encoding the enzymes of specified sequences above as well as specified deduced amino acid sequences, was initially amplified from a pBluescript vector containing the DNA by PCR technique. The restriction enzyme sites indicated parameters corresponding to the restriction enzyme sites on the bacterial expression. The pQE vector encoded antibiotic resistance, bacterial origin of replication, IPTG-regulatable promoter operator, ribosome binding site, 6His tag and restriction enzymes sites. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight in liquid culture. The cells were grown to an optical density of 0.4-0.6 Isopropyl-B-D thiogalacto pyranoside (IPTG) that was then added to the final concentration. The IPTG induced by inactivating the lad repressor, clearing the promoter operator leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by centrifugation. (53 pages)

L37 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2003 ACS

AB The invention provides 3 human cyclic nucleotide-assocd. proteins (CNAP) and polynucleotides which identify and encode CNAP. CNAP-1 has chem. and structural similarity with Saccharomyces kluyveri adenylyl cyclase, CNAP-2 is similar to Aquifex pyrophilus esterase 28LC, and CNAP-3 is similar to human sol. guanylate cyclase large subunit. Protein motifs, tissue specificity, and disease assocn. of the 3 CNAP proteins are also provided. The invention also provides expression vectors, host cells, antibodies, and antagonists. The invention also provides methods for diagnosing, treating or preventing cell proliferative, autoimmune/inflammatory, neurol., vision, reproductive, and smooth muscle disorders.

L37 ANSWER 3 OF 4 MEDLINE

The recJ gene, identified in Escherichia coli, encodes a Mg(+2)-dependent AB 5'-to-3' exonuclease with high specificity for single-strand DNA. Genetic and biochemical experiments implicate RecJ exonuclease in homologous recombination, base excision, and methyl-directed mismatch repair. Genes encoding proteins with strong similarities to RecJ have been found in every eubacterial genome sequenced to date, with the exception of Mycoplasma and Mycobacterium tuberculosis. Multiple genes encoding proteins similar to RecJ are found in some eubacteria, including Bacillus and Helicobacter, and in the archaea. Among this divergent set of sequences, seven conserved motifs emerge. We demonstrate here that amino acids within six of these motifs are essential for both the biochemical and genetic functions of E. coli RecJ. These motifs may define interactions with Mg(2+) ions or substrate DNA. A large family of proteins more distantly related to RecJ is present in archaea, eubacteria, and eukaryotes, including a hypothetical protein in the MgPa adhesin operon of Mycoplasma, a domain of putative polyA polymerases in Synechocystis and Aquifex, PRUNE of Drosophila, and an exopolyphosphatase (PPX1) of Saccharomyces cereviseae. Because these six RecJ motifs are shared between exonucleases and exopolyphosphatases, they may constitute an ancient phosphoesterase domain now found in all kingdoms of life.

L37 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI A new DNA or RNA nucleic acid molecule encoding an enzyme (DNA sequence AΒ and protein sequence specified) can be contained on a vector and used to transform a host cell for production of the recombinant protein. The enzyme is preferably an esterase, able to transfer an amino group from an amino acid to an alpha-keto acid, and may be useful in e.g. cheese or paper manufacture or to study plant resistance to disease, etc. Esterases are used to convert esters to organic acids and alcohols. The claimed enzymes are thermostable and stable in organic solvents, so are well suited to industrial operations. The DNA is derived from Staphylococcus marinus F1-12LC, Pyrodictium sp. TAG17-17LC, Archaeoglobus venificus SNP6-24LC, Aquiflex pyrophilus 28LC and M11TL-29L, Thermococcus sp. CL-2-30LC, Aquiflex sp. VF5-34LC, Teredinibacter sp. 44L, Archaeoglobus fulgidus VC16-16MC and Sulfolobus solfataricus P1-8LC, prepared by amplification of genomic DNA using DNA primers (sequence specified). The nucleic acid encoding the esterases can be used as DNA probes or RNA probes to identify related sequences. (112pp)

L38 30 L13(8A)GENE/Q

FILE 'SCISEARCH'
L39 35 L14(8A)GENE/Q

FILE 'LIFESCI'
L40 26 L15(8A)GENE/Q

FILE 'BIOTECHDS'
L41 9 L16(8A)GENE/Q

FILE 'BIOSIS'
L42 50 L17(8A)GENE/Q

FILE 'EMBASE'
L43 28 L18(8A)GENE/Q

96 L19(8A)GENE/Q

=> s 124(8a)gene/q
FILE 'MEDLINE'

FILE 'HCAPLUS'

L44

FILE 'NTIS'

L45 0 L20(8A)GENE/Q

FILE 'ESBIOBASE'

L46 27 L21(8A)GENE/Q

FILE 'BIOTECHNO'

L47 30 L22(8A)GENE/Q

FILE 'WPIDS'

L48 7 L23(8A)GENE/Q

TOTAL FOR ALL FILES

L49 338 L24(8A) GENE/Q

=> s 149 not 2002-2003/py

FILE 'MEDLINE'

732373 2002-2003/PY

L50 25 L38 NOT 2002-2003/PY

FILE 'SCISEARCH'

1279547 2002-2003/PY

L51 26 L39 NOT 2002-2003/PY

FILE 'LIFESCI'

100291 2002-2003/PY

L52 21 L40 NOT 2002-2003/PY

FILE 'BIOTECHDS'

27195 2002-2003/PY

L53 6 L41 NOT 2002-2003/PY

FILE 'BIOSIS'

647654 2002-2003/PY

L54 35 L42 NOT 2002-2003/PY

FILE 'EMBASE'

592164 2002-2003/PY

L55 20 L43 NOT 2002-2003/PY

FILE 'HCAPLUS'

1444038 2002-2003/PY

L56 63 L44 NOT 2002-2003/PY

FILE 'NTIS'

13299 2002-2003/PY

L57 0 L45 NOT 2002-2003/PY

FILE 'ESBIOBASE'

377784 2002-2003/PY

L58 20 L46 NOT 2002-2003/PY

FILE 'BIOTECHNO'

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L59 22 L47 NOT 2002-2003/PY

FILE 'WPIDS'

1410387 2002-2003/PY

L60 2 L48 NOT 2002-2003/PY

TOTAL FOR ALL FILES

L61 240 L49 NOT 2002-2003/PY

=> dup rem 161 PROCESSING COMPLETED FOR L61 L62 74 DUP REM L61 (166 DUPLICATES REMOVED) => d tot L62 ANSWER 1 OF 74 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI ΤI Thermostable DNA polymerase gene isolated from aquifex pyrophilus and its amino acid sequence; enzyme gene production and mofidication from bacterium ΑIJ CHOI J J; KWON S T AN 2002-16315 BIOTECHDS PΤ KR 2001113228 28 Dec 2001 L62 ANSWER 2 OF 74 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI TIAquifex pyrophilus derived thermostable pyrophosphatase (ApyPpase) and coding gene used for genetic engineering and diagnosis of hereditary diseases; vector plasmid pAPYP-mediated recombinant protein gene transfer and expression in Escherichia coli for use in disease diagnosis AU HUH H S; KWON S T AN 2002-14424 BIOTECHDS KR 2001097348 8 Nov 2001 ANSWER 3 OF 74 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI L62 TIGene encoding heat resistant alanine racemase of Aquifex pyrophilus, useful in medicine and food industry; enzyme production, vector expression in bacterium, fermentation and incubation for food industry and medicine ΑU YOO Y G AN 2002-08972 BIOTECHDS PΤ KR 2001083959 6 Sep 2001 L62 ANSWER 4 OF 74 MEDLINE DUPLICATE 2 Cytochromes c555 from the hyperthermophilic bacterium Aquifex aeolicus. 2. TT Heterologous production of soluble cytochrome c555s and investigation of the role of methionine residues. SO BIOCHEMISTRY, (2001 Nov 13) 40 (45) 13690-8. Journal code: 0370623. ISSN: 0006-2960. ΑU Aubert C; Guerlesquin F; Bianco P; Leroy G; Tron P; Stetter K O; Bruschi M AN 2001644001 MEDLINE L62 ANSWER 5 OF 74 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE ΤI Cytochromes c555 from the hyperthermophilic bacterium Aquifex aeolicus (VF5). 1. Characterization of two highly homologous, soluble and membranous, cytochromes c555. SO Biochemistry, (November 13, 2001) Vol. 40, No. 45, pp. 13681-13689. print. ISSN: 0006-2960. ΑU Baymann, Frauke; Tron, Pascale; Schoepp-Cothenet, Barbara (1); Aubert, Corinne; Bianco, Pierre; Stetter, Karl-Otto; Nitschke, Wolfgang; Schutz, Michael AN2001:567239 BIOSIS L62 ANSWER 6 OF 74 HCAPLUS COPYRIGHT 2003 ACS Genes linked by fusion events are generally of the same functional TΙ category: a systematic analysis of 30 microbial genomes SO Proceedings of the National Academy of Sciences of the United States of America (2001), 98(14), 7940-7945 CODEN: PNASA6; ISSN: 0027-8424 Yanai, Itai; Derti, Adnan; DeLisi, Charles

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136:178666

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- SO Journal of Bacteriology, (November, 2001) Vol. 183, No. 22, pp. 6565-6572. print.
 ISSN: 0021-9193.
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- AN 2001:540050 BIOSIS
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- TI Comprehensive comparison between locations of orthologous genes on archaeal and bacterial genomes
- SO Bioinformatics (2001), 17(9), 791-802 CODEN: BOINFP; ISSN: 1367-4803
- AU Horimoto, Katsuhisa; Fukuchi, Satoshi; Mori, Kentaro
- AN 2001:813196 HCAPLUS
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- L62 ANSWER 9 OF 74 MEDLINE DUPLICATE 4
- TI Identification and cloning of partial mbh2 gene cluster of hyperthermophile Aquifex pyrophilus.
- SO WEI SHENG WU HSUEH PAO [ACTA MICROBIOLOGICA SINICA], (2001 Dec) 41 (6) 674-9.
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- AN 2003044208 IN-PROCESS
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- DN 135:104920
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- TI Phylogenetic analyses of two "archaeal" genes in Thermotoga maritima reveal multiple transfers between archaea and bacteria
- SO Molecular Biology and Evolution (2001), 18(3), 362-375 CODEN: MBEVEO; ISSN: 0737-4038
- AU Nesbo, Camilla L.; L'Haridon, Stephane; Stetter, Karl O.; Doolittle, W. Ford

DUPLICATE 5

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- L62 ANSWER 12 OF 74 MEDLINE
- TI Prokaryotic structural maintenance of chromosomes (SMC) proteins: distribution, phylogeny, and comparison with MukBs and additional prokaryotic and eukaryotic coiled-coil proteins.
- SO GENE, (2001 Oct 31) 278 (1-2) 253-64. Journal code: 7706761. ISSN: 0378-1119.
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- TI Expression in Escherichia coli of the thermostable inorganic pyrophosphatase from the Aquifex aeolicus and purification and characterization of the recombinant enzyme.
- SO PROTEIN EXPRESSION AND PURIFICATION, (2001 Nov) 23 (2) 242-8. Journal code: 9101496. ISSN: 1046-5928.
- AU Hoe H S; Kim H K; Kwon S T

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- TI Molecular cloning and characterization of thermostable DNA ligase from Aquifex pyrophilus, a hyperthermophilic bacterium.
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- TI Genome of Aquifex aeolicus
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 CODEN: MENZAU; ISSN: 0076-6879
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- SO FEMS Microbiology Letters (2001), 202(1), 115-119 CODEN: FMLED7; ISSN: 0378-1097
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- SO FEMS MICROBIOLOGY LETTERS, (2001 Jul 10) 201 (1) 73-7. Journal code: 7705721. ISSN: 0378-1097.
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- TI Evolution of gene order conservation in prokaryotes
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- AN 2002:227287 HCAPLUS
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- SO U.S., 17 pp. CODEN: USXXAM
- IN Han, Ye Sun; Yu, Yeon Gyu; Kim, Sung Hou; Lim, Jae Hwan; Ryu, Jae Ryeon; Choi, In Geol
- AN 2000:157663 HCAPLUS
- DN 132:204860
- PATENT NO. KIND DATE APPLICATION NO. DATE

 PI US 6033889 A 20000307 US 1998-8303 19980116
- L62 ANSWER 20 OF 74 HCAPLUS COPYRIGHT 2003 ACS
- TI Cyclic glucan synthesis with thermostable branching enzyme for use in food production
- SO Jpn. Kokai Tokkyo Koho, 12 pp. CODEN: JKXXAF

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PATENT NO. KIND DATE APPLICATION NO. DATE

PI JP 2000316581 A2 20001121 JP 1999-130833 19990512

- L62 ANSWER 21 OF 74 HCAPLUS COPYRIGHT 2003 ACS
- TI Protein and cDNA sequences for a human ADP- ribosylglycohydrolase protein ARGHase, its expression and use
- SO Faming Zhuanli Shenqing Gongkai Shuomingshu, 21 pp. CODEN: CNXXEV
- IN Li, Nenggan; Qian, Binzhi; Gao, Xin; Xiao, Huasheng; Chen, Zhu; Han, Zequang
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PATENT NO. KIND DATE APPLICATION NO. DATE

CN 1264741 A 20000830 CN 2000-111778 20000302

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- TI Antibacterial agents that target lipid A biosynthesis in gram-negative bacteria: inhibition of diverse UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylases by substrate analogs containing zinc binding motifs
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- L62 ANSWER 23 OF 74 MEDLINE DUPLICATE 9
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- SO JOURNAL OF BACTERIOLOGY, (2000 Aug) 182 (15) 4278-87. Journal code: 2985120R. ISSN: 0021-9193.
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- TI Cloning and characterization of thermostable endoglucanase (Cel8Y) from the hyperthermophilic Aquifex aeolicus VF5.
- SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2000 Dec 20) 279 (2) 420-6.

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- AN 2001111040 MEDLINE
- L62 ANSWER 26 OF 74 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 12
- TI Phylogenetic depth of the bacterial genera Aquifex and Thermotoga inferred from analysis of ribosomal protein, elongation factor, and RNA polymerase subunit sequences.
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- AN 2000:241251 BIOSIS
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- TI Hyperthemostable AONS: The first committed enzyme in E. coli biotin biosynthesis has a tropical relative.
- SO Biochemical Society Transactions, (October, 2000) Vol. 28, No. 5, pp. A316. print.

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- Polypurine.polypyrimidine sequences in complete bacterial genomes: preference for polypurines in protein-coding regions
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- AN 2000:154073 HCAPLUS
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- L62 ANSWER 29 OF 74 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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- SO Protein Expression and Purification, (April, 2000) Vol. 18, No. 3, pp. 257-261.
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- AN 2000:233620 BIOSIS
- L62 ANSWER 30 OF 74 LIFESCI COPYRIGHT 2003 CSA DUPLICATE 13
- TI Reverse gyrase from hyperthermophiles: Probable transfer of a thermoadaptation trait from Archaea to Bacteria
- SO Trends in Genetics [Trends Genet.], (20000400) vol. 16, no. 4, pp. 152-156.
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- AU Forterre, P.; De La Tour, C.B.; Philippe, H.; Duguet, M.
- AN 2000:76087 LIFESCI
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- L62 ANSWER 32 OF 74 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 15
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- SO ENZYME AND MICROBIAL TECHNOLOGY, (JUL 2000) Vol. 27, No. 1-2, pp. 83-88. Publisher: ELSEVIER SCIENCE INC, 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010.
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YOU HAVE REQUESTED DATA FROM 9 ANSWERS - CONTINUE? Y/(N):y

- L62 ANSWER 66 OF 74 HCAPLUS COPYRIGHT 2003 ACS
- Isolated nucleic acids are provided which encode a thermostable protein which binds specifically to bulge loops in a heteroduplex nucleic acid and recombinant vectors comprising nucleic acid which encodes a thermostable protein which binds specifically to bulge loops in a heteroduplex nucleic acid. MutS genes were cloned into Escherichia coli from 2 distantly related hyperthermophilic eubacteria, Aquifex pyrophilus and Thermotoga maritima, based on PCR technol. without the need for library construction. Based on the binding activity for bulge loops in a heteroduplex, the recombinant MutS proteins can reduce DNA misincorporation in an amplification reaction, and thus be used in methods for detecting a nucleic acid which includes a specific sequence, amplifying a nucleic acid comprising a specific sequence, and selecting against a nucleic acid comprising a specific sequence. Allele-specific amplification with matched primers demonstrates that MutS binding to a variety of mismatched primer-template complexes inhibits initiation of polymn. With mismatched internal oligonucleotide, propagation of polymn. can be inhibited by forming a MutS-internal duplex mismatch complex; MutS-mediated selective amplification occurs at each PCR cycle, if needed.
- L62 ANSWER 67 OF 74 MEDLINE DUPLICATE 29
- AB A poly(U)-programmed cell-free system from the hyperthermophilic bacterium Aquifex pyrophilus has been developed, and the susceptibility of Aquifex ribosomes to the miscoding-inducing and inhibitory actions of all known classes of aminoglycoside antibiotics has been assayed at temperatures (75 to 80 degrees C) close to the physiological optimum for cell growth. Unlike Thermotoga maritima ribosomes, which are systematically refractory to all known classes of aminoglycoside compounds (P. Londei, S.

Altamura, R. Huber, K. O. Stetter, and P. Cammarano, J. offteriol. 170-4353-4360, 1988), Aquifex ribosomes are susceptible to all of the aminoglycosides tested (disubstituted 2-deoxystreptamines, monosubstituted 2-deoxystreptamines, sand streptidine compounds). The significance of this result in light of the **Aquifex** and Thermotoga placements in phylogenetic trees of molecular **sequences** is discussed.

DUPLICATE 30 ANSWER 68 OF 74 MEDLINE The polytrichously inserted flagella of Aquifex pyrophilus, a marine AΒ hyperthermophilic bacterium growing at 85 degrees C, were isolated and purified. Electron micrographs of the 19-nm-diameter flagellar filaments show prominent helical arrays of subunits. The primary structure of these 54-kDa flagellin monomers determining the helical shape and heat stability of filaments was of particular interest. The genomic region encoding the flagellin subunit (flaA gene) and an upstream open reading frame (orf1) were cloned and sequenced. The 1,503-bp flaA and 696-bp orf1 are preceded by separate sigma 28-like promoters and ribosome-binding motifs and succeeded by palindromic transcription terminators. Both genes are actively transcribed, but the nature and function of the orf1-encoded 231-residue polypeptide remain unknown. The deduced primary structure of the 501-amino-acid flagellin encoded by flaA consists of conserved N- and C-terminal regions and a variable 246-residue central domain. In comparison to mesophilic flagellins, the thermostable A. pyrophilus flagellin is characterized by increases in aromatic residues and prolines as well as by a 7.9% +/- 3.2% increase in all hydrophobic residues that is balanced by a respective decrease in hydrophilic residues. This composition is thought to form more compact flagellin monomers and stable interface contacts between neighboring subunits in the polymer.

L62 ANSWER 69 OF 74 HCAPLUS COPYRIGHT 2003 ACS Universal trees based on sequences of single gene homologs cannot be rooted. Iwabe et al. [Iwabe, N., Kuman, K.-I., Hasegawa, M., Osawa, S. and Miyata, T. (1989) Proc. Natl. Acad. Sci. USA 86, 9355-9359] circumvented this problem by using ancient gene duplications that predated the last common ancestor of all living things. Their sep., reciprocally rooted gene trees for elongation factors and ATPase subunits showed Bacteria (eubaceria) as branching first from the universal tree with Archea (archaebacteria) and Eucarya (eukaryotes) as sister groups. Given its topical importance to evolutionary biol. and concerns about the appropriateness of the ATPase data set, an evaluation of the universal tree root using other ancient gene duplications is essential. In this study, we derive a rooting for the universal tree using aminoacyl-tRNA synthetase genes, an extensive multigene family whose divergence likely preceded that of prokaryotes and eukaryotes. An approx. 1600-bp conserved region was sequenced from the isoleucyl-tRNA synthetases of several species representing deep evolutionary branches of eukaryotes (Nosema locustae), Bacteria (Aquifex pyrophilus and Thermotoga maritima) and Archea (Pyrococcus furiosus and Sulfolobus acidocaldarius). In addn., a new valyl-tRNA synthetase was characterized from the protist Trichomonas vaginalis. Different phylogenetic methods were used to generate trees of isoleucyl-tRNA synthetases rooted by valyl- and leucyl-tRNA synthetases. All isoleucyl-tRNA synthetase trees showed Archaea and Eucarya as sister groups, providing strong confirmation for the universal tree rooting reported by Iwabe et al. As well, there was strong support for the monopoly (sensu Hennig) of Archea. The valyl-tRNA synthetase gene from Tr. vaginalis clustered with other eukaryotic VaIRS genes, which may have been transferred from the mitochondrial genome to the nuclear genome, suggesting that this amitochondrial trichomonad once harbored and endosymbiotic bacterium.

L62 ANSWER 70 OF 74 MEDLINE DUPLICATE 31

AB The gene fus (for EF-G) of the hyperthermophilic bacterium

Aquifex pyrophilus was cloned and sequenced.

Unlike the other bacteria, which display the streptomycin-operon

arrangement of EF genes (5'-rps12-rps7-fus-tuf-3'), the Aquifex fus gene (700 codons) is not preceded by the two small ribosomal subunit genes although it is still followed by a tuf gene (for EF-Tu). The opposite strand upstream from the EF-G coding locus revealed an open reading frame (ORF) encoding a polypeptide having 52.5% identity with an E. coli protein (the pdxJ gene product) involved in pyridoxine condensation. The Aquifex EF-G was aligned with available homologs representative of Deinococci, high G+C Gram positives, Proteobacteria, cyanobacteria, and several Archaea. Outgroup-rooted phylogenies were constructed from both the amino acid and the DNA sequences using first and second codon positions in the alignments except sites containing synonymous changes. Both datasets and alternative tree-making methods gave a consistent topology, with Aquifex and Thermotoga maritima (a hyperthermophile) as the first and the second deepest offshoots, respectively. However, the robustness of the inferred phylogenies is not impressive. The branching of Aquifex more deeply than Thermotoga and the branching of Thermotoga more deeply than the other taxa examined are given at bootstrap values between 65 and 70% in the fus-based phylogenies, while the EF-G(2)-based phylogenies do not provide a statistically significant level of support (< or = 50% bootstrap confirmation) for the emergence of Thermotoga between Aquifex and the successive offshoot (Thermus genus). At present, therefore, the placement of Aquifex at the root of the bacterial tree, albeit reproducible, can be asserted only with reservation, while the emergence of Thermotoga between the Aquificales and the Deinococci remains (statistically) indeterminate.

- L62 ANSWER 71 OF 74 HCAPLUS COPYRIGHT 2003 ACS
- Sequences of the recA genes of the highly divergent thermophilic eubacteria Thermus aquaticus (and Thermus thermophilus), Thermotoga maritima, and Aquifex pyrophilus were detd. from fragments derived by polymerase chain reaction (PCR) with degenerate primers and from inverse PCR products obtained using unique primers based on the fragment sequences. The source of the PCR products was verified by Southern hybridization. Complete PCR-derived recA genes were cloned into an expression vector regulated by a temp.-sensitive .lambda.-repressor, and independently derived clones expressing thermostable RecA were selected. DNA sequences were verified to be authentic by direct cycle-sequencing of PCR products and/or sequencing of several clones. In contrast to Escherichia coli RecA protein, all the purified thermophilic RecA proteins exhibited single-stranded DNA-dependent ATPase activity optima above 70 .degree.C. Phylogenetic anal. of RecA sequences suggested that the thermophilic RecA proteins were at least as different from one another as were Gram-pos. organisms, mesophilic Gram-neg. organisms, and cyanobacteria. In spite of substantial sequence divergence, interesting characteristics of the thermostable RecA proteins included increased valine content, common amino acid replacements at two highly conserved sites, and an increase in the calcd. isoelec. point of approx. a full pH unit.
- L62 ANSWER 72 OF 74 HCAPLUS COPYRIGHT 2003 ACS
- AB A genomic map of the hyperthermophilic hydrogen-oxidizing bacterium Aquifex pyrophilus was established with NotI (GC/GGCCGC), SpeI (A/CTAGT), and XbaI (T/CTAGA). Linking clones and cross-hybridization of restriction fragments revealed a single circular chromosome of 1.6 Mbp. A single flagellin gene and six rRNA gene units were located on this map by Southern hybridization.
- ANSWER 73 OF 74 MEDLINE DUPLICATE 32

 The phylogenetic diversity of a well-known pink filament community associated with the 84 to 88 degrees C outflow from Octopus Spring, Yellowstone National Park, was examined. Three phylogenetic types ("phylotypes"), designated EM 3, EM 17, and EM 19, were identified by cloning and sequencing the small subunit rRNA genes (16S rDNA) obtained by PCR amplification of mixed-population DNA. All three phylotypes diverge

deeply within the phylogenetic domain Bacteria sensu Woese (C. R. Woese, O. Kandler, and M. L. Wheelis, Proc. Natl. Acad. Sci. USA 87:4576-4579, 1990). No members of the Archaea or Eucarya were detected. EM 3 comprises a unique lineage within the Thermotogales group, and EM 17 and EM 19 are affiliated with the Aquificales. A total of 35 clones were examined, of which the majority (26 clones) were of a single sequence type (EM 17) closely related to Aquifex pyrophilus. In situ hybridization with clone-specific probes attributes the majority sequence, EM 17, to the pink filaments.

ANSWER 74 OF 74 MEDLINE DUPLICATE 33

The 16S rRNA of the bacterion Aquifex pyrophilus, a microaerophilic, oxygen-reducing hyperthermophile, has been sequenced directly from the the PCR amplified gene. Phylogenetic analyses show the Aq. pyrophilus lineage to be probably the deepest (earliest) in the (eu)bacterial tree. The addition of this deep branching to the bacterial tree further supports the argument that the Bacteria are of thermophilic ancestry.

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